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WORLD REFERENCE CENTER FOR ARBOVIRUSES

FINAL REPORT

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SUMMARY:

The World Reference Center for Arboviruses maintains on a national and an international basis: a) serologic identification and biologic characterization of arboviruses using CF, HI, neutralization, IFA, and ELISA techniques and reagents prepared in large part in mice; b) molecular characterization of viruses using PAGE, electron microscopy, and polypeptide and RNA purification; c) diagnosis of disease using sera and other specimens submitted by the military and other organizations; d) diagnosis and epidemiological study of epidemics and epizootics using submitted specimens; e) preparation and distribution of reference reagents including antibody, viruses to specific organizations, and antigens under special circumstances; f) serological survey for arboviruses on a limited scale; and g) dissemination of information through WHO and the American Committee on Arthropod-borne Viruses. The above functions of the reference center were jointly supported by this grant and by grants from the U.S. NIH, the Australian government, and WHO.

During the 3 years of this grant, 197 viruses were identified, many of them new to science and/or new to a geographic region. The taxonomy of the rhabdoviruses, orbiviruses, and phleboviruses was revised. The distribution of antibody to arboviruses was determined throughout specific areas of the world by serosurvey. Techniques for rapid and early diagnosis of arbovirus diseases were refined and transferred as technology to users internationally. Two thousand, two hundred fifty-eight ampoules of arbovirus reagents were distributed to the U.S. Military and to other laboratories throughout the world.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985).

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

BODY OF REPORT:

Introduction. The World Reference Center for Arboviruses was established at the Yale Arbovirus Research Unit in 1965 as an outgrowth of The Rockefeller Foundation program on arboviruses which was moved in 1965 to Yale University from New York City. The U.S. Army has supported this program since 1972, initially through joint Navy-Army funding, then through a separate contract, and during the past three years by this grant. The progress of the past three years is included in this report; it covers the work for the entire project which received support from WHO, NIH, and the Australian government, in addition to that of this grant.

Virus identification. A primary function of the reference center is to receive viruses from all parts of the world for identification. Attempts to identify about 200 isolates in mosquito cells from Indonesia determined that at least 64 of these were doublestranded segmented RNA viruses. Eleven of these had 9 segments, 8 had 10 segments in a pattern of 2-2-2-2-2, 11 had 10 segments similar to Matsu virus 2-4-3-1, one had 10 segments 3-3-3-1, 6 were EHD-like, 16 had 10 segments 3-6-1, 10 had 12 segments in a pattern 6-6, and one had 12 segments in a pattern 6-5-1. Several appeared to be mixtures with 3- or 5-segmented viruses as the second virus. Ten also reacted with the flavivirus grouping antibody, and 3 with alphavirus grouping antibody. Another Indonesian NAMRU isolate was a rhabdovirus, apparently new to science.

Viruses were identified from the United States and from many other nations as follows:

ALPHAVIRUSES. Chikungunya virus was identified from Culex mosquitoes from Central African Republic, and several strains of Highlands J virus were identified from Connecticut.

FLAVIVIRUSES. Dengue-4 was recognized from New Caledonia, several strains of Japanese encephalitis from Viet Nam, and a flavivirus from ticks of Connecticut (possibly a contaminant).

BUNYAVIRUSES. A new virus from ticks from India was in the Tete serogroup. Inkoo virus was identified for the first time from Sweden. A new Simbu group virus was recognized from Brazil and for the first time, a Bunyamwera serogroup virus was identified from Australia.

PHLEBOVIRUSES. New phleboviruses were characterized from Brazil and Colombia; Chagres was isolated for the first time from Colombian sandflies; and Toscana virus was identified from the cerebrospinal fluid of a tourist visiting in Portugal. Naples virus was identified, isolated by NAMRI-3 personnel in Egypt, and Sicilian and Naples-like viruses were identified from Swedish UN troops.

NAIROVIRUSES. The Xinjiang hemorrhagic fever virus from western China was identified as Crimean-Congo hemorrhagic fever virus. A strain of Avalon virus was found in Canada and a new nairovirus from a Brazilian bird was identified.

UUKUVIRUSES. Three new Uukuniemi serogroup viruses were characterized, Precarious Point, murre, and an as yet unnamed Alaskan isolate.

VESICULOVIRUSES. New VSV-related rhabdoviruses were recognized from Argentina and the United States. Also, a Tibrogargan-related rhabdovirus from Florida was characterized.

ORBIVIRUSES. In addition to the orbiviruses described above from Indonesia, several isolates of tick-borne (Kemerovo serogroup) viruses were identified from Canada, Australia, Finland, France, and California.

ARENAVIRUSES. Confirmation of the relationship of Ippy virus to Lassa virus was made. This finding was first reported from the Institute of Virology in South Africa.

UNCLASSIFIED VIRUSES. Several ungrouped and unclassified viruses were studied including Lake Clarendon virus from Australia, a virus from New York State voles, tick agents from western USA and from Tanzania, and a new mosquito isolate from Brazil.

Classification of arboviruses. The Quaranfil serogroup was characterized by electron microscopy as arenavirus-like. The serological relationships of a new member of the group were determined, and six proteins were found by PAGE analysis of infected cells. No serological relationship was found to other arenaviruses.

The members of the VSV serogroup were characterized by ELISA, neutralization, CF and immunofluorescence. The study included two new vesiculoviruses.

A comprehensive study of the orbiviruses was carried out including members of the bluetongue, EHD, Palyam, Corriparta, Kemerovo, and Colorado tick fever serogroups. The study embraced PAGE analyses and RNA-RNA blot hybridization. Extensive attempts to produce reassortants among orbiviruses showed that members of the serogroups reassorted, but that members of different serogroups did not reassort, even under extreme conditions. It was proposed that viruses which reassort are members of a single species, and that therefore viruses in a serogroup constituted a species. The RNA-RNA blot hybridization was demonstrated to be a sensitive and reliable identification method.

Diagnosis of disease. Sandfly fever was diagnosed in Swedish soldiers in Cyprus; EEE was confirmed in Whooping Cranes in Maryland; and equine, human, and avian encephalitis was identified in Connecticut.

Serologic and antigen surveys. Rift Valley fever antibody was shown in an indigenous population inhabiting the Senegal River Valley at the site near the river mouth where the Diama Dam has been constructed. Antibody to RVF was also shown in Zambia in farm residents who survived a hemorrhagic fever outbreak. The ELISA was used also to show widespread RVF antibody in sheep

and cattle from Mali. Antigen and antibody to a Hantaan-related virus was detected in voles from Connecticut. An antibody survey of Uganda revealed alpha-, flavi-, and bunyavirus reactivity.

Characterization of monoclonal antibodies. Sandfly Sicilian monoclonal antibodies supplied by scientists at Fort Detrick were characterized for their reactivity by immunofluorescence with a battery of phleboviruses.

Development of new techniques. The 4G2-4-15 dengue-2 monoclonal antibody was developed and used extensively as a universal flavivirus capture antibody in ELISA. Grouping monoclonal antibodies from CDC Fort Collins and from the University of Maryland were characterized and shown to be highly group reactive by HI and ELISA. The ELISA using SDS treated orbivirus antigen coat was developed for testing seabirds for Kemerovo group antibodies. An ELISA was elaborated for detecting Crimean-Congo hemorrhagic fever antibody in sheep, cattle, and humans. The technique was tested in Israel where CCHF antibody was found for the first time there in cattle. The enzyme immunoassay was adapted to use dengue virus-infected cultured mosquito cells as antigen and an arbovirus tissue culture neutralization test was developed using ELISA as an indicator. Mice immunized by intrasplenic inoculation with arboviruses developed ELISA antibody in 5 days. This method allows rapid reciprocal definitive identification of arboviruses.

Experimental pathogenesis. Hamsters were used as a model for testing viremia and immune response to sequential infections with phleboviruses. Immune enhancement was not shown, but broadening of immune response following sequential infections with different phleboviruses was evident.

Collection of low passage arbovirus strains. A large collection of low passage arbovirus strains was developed and maintained lyophilized. Priority was given to yellow fever, dengue, chikungunya, California encephalitis, Venezuelan encephalitis, St. Louis encephalitis, western encephalitis, eastern encephalitis, Japanese encephalitis, and other human disease arboviruses. Vesiculovirus strains of veterinary importance were also accessioned. The original (or as close to original as was available) material was passaged once in C6/36 mosquito cells or in Vero cells. The resulting stock was lyophilized in aliquots. These were stored and distributed to any and all persons requesting material for study.

Distribution of reagents. Reference antibodies and viruses were maintained as lyophilized stock for distribution to U.S. military users and to others on a world-wide basis. During the three years of the grant, 2,258 ampoules of arbovirus reagents were distributed to laboratories in more than 22 countries. This total consisted of 627 ampoules of virus stock, 1,055 ampoules of virus antigen, and 576 ampoules of antibody. The reagents distributed represented more than 200 different arboviruses.

Eight different cell lines were also distributed. These included both vertebrate and mosquito cells.

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